



5fpo-02-34 amended sequence listing

<110> Korea Research Institute of Bioscience and Biotechnology
<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase
<130> 26666U
<150> KR 2002-55575
<151> 2002-09-13
<160> 19
<170> KopatentIn 1.71
<210> 1
<211> 27
<212> DNA
<213> Artificial Sequence
<220>
<223> CALB primer 1

<400> 1
ggctcttcag ccactccttt ggtgaag 27

<210> 2
<211> 23
<212> DNA
<213> Artificial Sequence
<220>
<223> CALB primer 2

<400> 2
gcggatcctc agggggtgac gat 23

<210> 3
<211> 27
<212> DNA
<213> Artificial Sequence
<220>
<223> CALB primer 3

<400> 3
gcggatccgg ggggtgacgat gccggag 27

<210> 4
<211> 19
<212> DNA
<213> Artificial Sequence
<220>
<223> GPD-err primer

<400> 4

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gcagagctaa ccaataagg

19

<210> 5
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T-0 primer

<400> 5
 tgcagttgaa cacaaccac

19

<210> 6
 <211> 1023
 <212> DNA
 <213> Candida antarctica

<220>
 <221> sig_peptide
 <222> (1)..(51)
 <223> secretion signal

<400> 6
 atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtag cgccactccc 60
 ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 120
 gatgcgggtc tgacctgcca ggggtgcttcg ccatactcgg tctccaaacc catccttctc 180
 gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctctt 240
 gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcatgct caacgacacc 300
 cagggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360
 aacaagcttc ccgtgctcac ctgggtccag ggtggtcttg ttgcacagtg ggggtctgacc 420
 ttcttcccca gtatcaggtc caaggctgat cgacttatgg cttttgcgcc cgactacaag 480
 ggcaccgtcc tcgccggccc tctcgatgca ctcgcggtta gtgcaccctc cgtatggcag 540
 caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtggtct gaccagatc 600
 gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660
 tcgccactcg actatccta cctcttcaac gggaagaacg tccaggcaca ggctgtgtgt 720
 gggccgctgt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780
 ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacc 840
 gactgcaacc ctcttcccg ccaatgatctg actcccgagc aaaaggctcg cgcggtgctg 900
 ctcccggtgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgcg 960
 ctcatgccct acgcccggcc ctttgtagta ggcaaaagga cctgctccgg catcgtcacc 1020
 ccc 1023

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<210> 7
 <211> 1023
 <212> DNA
 <213> Candida antarctica

<220>
 <221> sig_peptide
 <222> (1)..(51)
 <223> secretion signal

<400> 7
 atgaatatat tttacatatt tttgtttttg ctgtcattcg ttcaaggtag cgccactcct 60
 ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc 120
 gatgcggggtc tgacctgcca aggtgcttcg ccatcctcgg tctccaaacc catccttctc 180
 gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctctt 240
 gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc 300
 caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360
 aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg ggggtctgacc 420
 ttcttcccca gtatcagggtc caaggctgat cgacttatgg cttttgcgcc cgactacaag 480
 ggcaccgtcc tcgccggccc tctcgatgca ctgcggtta gtgcaccctc cgtatggcag 540
 caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gaccagatc 600
 gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660
 tcgccactcg actcatccta ctttttcaac ggaaagaacg tccaggcaca ggctgtgtgt 720
 gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780
 ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcggacta tggcattacg 840
 gactgcaacc ctcttccgc caatgatctg actcccgagc aaaaggctcg cgcggtcg 900
 ctccggcg cgcggtgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960
 ctcatgccct acgcccgc ctttgagta ggcaaaagga cctgctccgg catcgtcacc 1020
 ccc 1023

<210> 8
 <211> 1023
 <212> DNA
 <213> Candida antarctica

<220>
 <221> sig_peptide
 <222> (1)..(51)
 <223> secretion signal

<400> 8

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atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtac cgccactcct	60
ttggtgaagc gtctgccttc cgggttcggac cctgcctttt cgcagcccaa gtcggtgctc	120
gatgcgggtc tgacctgcca ggggtgcttcg ccattcctcg tctccaaacc catccttctc	180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctctt	240
gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgct caacgacacc	300
caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac	360
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggcttgacc	420
ttcttcccca gtatcagggtc caaggctgat cgacttatgg cttttgcgcc cgactacaag	480
ggcaccgtcc tcgccggccc tctcgatgca ctgcggtta gtgcaccctc cgtatggcag	540
caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtggtct gaccagatc	600
gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac	660
tcgccactcg actcatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt	720
gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc	780
ggctgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacg	840
gactgcaacc ctcttcccgc caatgatctg actcccagac aaaaggctgc cgcggctgcg	900
ctcctggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgc	960
ctcatgccct acgcccgcc ctttgcatga ggcaaaagga cctgctccgg catcgtcacc	1020
ccc	1023

<210> 9
 <211> 343
 <212> PRT
 <213> Candida antarctica

<220>
 <221> SIGNAL
 <222> (1)..(17)
 <223> secretion signal

<400> 9
 Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 1 5 10 15
 Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
 20 25 30
 Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
 35 40 45
 Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
 50 55 60
 Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
 65 70 75 80

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Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240
Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
290 295 300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335
Gly Ile Val Thr Pro Gly Ser
340

<210> 10
<211> 343
<212> PRT
<213> Candida antarctica
<220>
<221> SIGNAL
<222> (1)..(17)
<223> secretion signal

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<400>      10
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
 1          5          10          15
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
          20          25          30
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
          35          40          45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
          50          55          60
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
 65          70          75          80
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
          85          90          95
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
          100          105          110
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
          115          120          125
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
          130          135          140
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
          145          150          155          160
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
          165          170          175
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
          180          185          190
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
          195          200          205
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
          210          215          220
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
          225          230          235          240
Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
          245          250          255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
          260          265          270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
          275          280          285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
          290          295          300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
          305          310          315          320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

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325

330

335

Gly Ile Val Thr Pro Gly Ser
340

<210> 11
<211> 341
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (1)..(24)
<223> secretion signal

<400> 11
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

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Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro
290 295 300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335
Gly Ile Val Thr Pro
340

<210> 12
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> CALB primer 4

<400> 12
ctcatatgct accttccggt tcggac

26

<210> 13
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> a-amylase secretion signal

<400> 13
Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala
1 5 10 15
Ala Pro Ala Leu Ala
20

<210> 14
<211> 317
<212> PRT
<213> Candida antarctica

<400> 14
Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
1 5 10 15
Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

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Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45
Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60
Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80
Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95
Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110
Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125
Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140
Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160
Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175
Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190
Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205
Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220
Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240
Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255
Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270
Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
275 280 285
Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300
Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> LQ53 primer

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<400> 15
gctgtgtgtg ggccgcagtt cgtcatcg 28

<210> 16
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LQ35 primer

<400> 16
gcatggtcga tgacgaactg cggcccacac 30

<210> 17
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> LP53 primer

<400> 17
gtcgccgcgg ctgcgctccc ggcgccggcg 30

<210> 18
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> LP35 primer

<400> 18
ctgcagccgc cggcgccggg agcgagcc 29

<210> 19
<211> 343
<212> PRT
<213> Candida antarctica

<400> 19
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

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Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340